3783: Comparison of DNA and RNA input IGH Chain Sequencing Assays for Somatic Hypermutation Analysis

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ABSTRACT

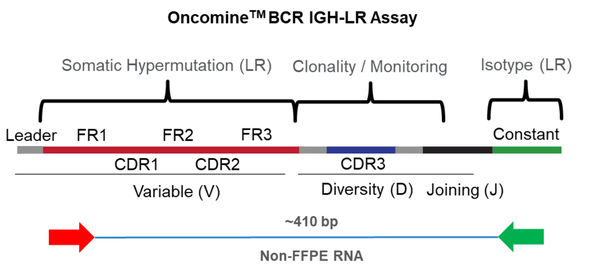
Current NGS sequencing methods for analyzing SHM rely on multiplex primers targeting the framework 1 (FR1) or Leader regions of the IGH variable gene and joining gene primers to amplify rearranged IGH chains from gDNA templates. Ion Ampliseq primer panels for SHM evaluation were compared using both DNA and RNA input. Performance was compared using SHM values obtained from RNA samples amplified using FR1 variable gene primers in combination with constant gene primers to determine each IGH isotype and subtype in a single PCR reaction. Comparison of SHM frequencies measured from matched RNA and DNA samples were used to determine the feasibility for use of RNA in the study of SHM as well as comparison of the performance of Leader and FR1 gene primers in DNA studies.

INTRODUCTION

Oncomine™ BCR IGH LR Assay and Oncomine™ Comprehensive IGHV SHM Assays (Leader-J/FR1-J)

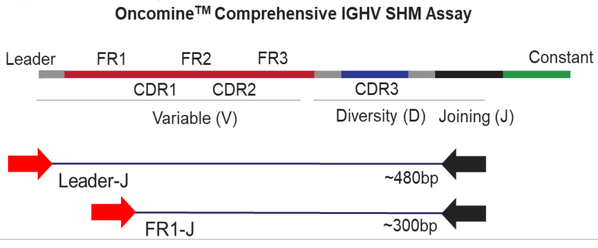
Assay Design covers CDR1, CDR2, CDR3, and CH1 domain of the constant gene with framework 1 and isotype-specific primers (FR1-C). This design enables accurate quantitation of somatic hypermutation, clonal expansion, isotype switching and identification of clonal lineages. Constant region primers are designed against all B cell isotypes and subtypes. Input requirements ranging from 25ng to 2ug of non-FFPE RNA.

Figure 1. Primer Design



Assay Design primers target the leader and FR1-J region in a separate reaction that can accurately measure clonal frequencies across B cell rearrangements. Input requirements range from 200ng to 2ug of FFPE DNA.

Figure 2. Primer Design



Qualifying SHM in Germline and CLL Research Samples

Internal Data Oncomine™ BCR-IGH LR libraries were prepared using plasmid constructs containing full length IGH chains cloned from germline and CLL research samples that were spiked into PBL total RNA background. These libraries were sequenced using the Ion™ GeneStudio S5 530 chip and analyzed using the Ion Reporter software to evaluate the ability to quantify somatic hypermutation, identify isotype, clonal structure of germline and CLL research samples.

Research Sample		Expected			Observed			
Accession	SHM Status	V-Gene SHM Frequency	Isotype	Clonal Structure	V-Gene SHM Frequency	Isotype	Clonal Structure	Status
JX432218.1	Mutated	0.037	IgA1	Monoclonal	0.048	IgA1	Monoclonal	PASS
AF021966.1	Mutated	0.088	IgG2	Monoclonal	0.102	IgG2	Monoclonal	PASS
AF021964.1	Mutated	0.084	IgG1	Monoclonal	0.088	IgG1	Monoclonal	PASS
JX432219.1	Mutated	0.058	IgA2	Monoclonal	0.057	IgA2	Monoclonal	PASS
JX432222.1	Germline	0	IgG3	Monoclonal	0	IgG3	Monoclonal	PASS
AF021958.1	Germline	0	IgM	Monoclonal	0	IgM	Monoclonal	PASS
AF021967.1	Germline	0	IgD	Monoclonal	0	IgD	Monoclonal	PASS

Table 1. Indicates observed SHM levels measured using Oncomine™ BCR-IGH LR Assay is comparable to known SHM frequencies from known CLL sequences which were designed into synthetic plasmid controls.

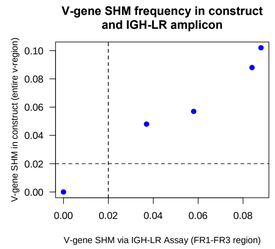
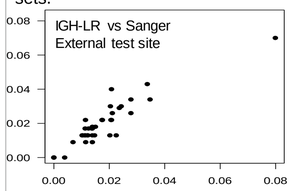


Figure 3. Measured vs Known SHM Frequencies Dashed lines indicate 2% SHM cutoff used to distinguish germline from CLL samples. Plot compares measured and known SHM frequency using control plasmids shown in Figure 3 . V-gene SHM frequencies for constructs are calculated over entire V-gene (including leader sequence).

Correlation between Ion Oncomine™ BCR IGH LR Assay vs Sanger Sequencing

External Data Oncomine™ BCR-IGH LR SHM values were compared to those obtained by Sanger sequencing using IGH-Leader or FR1 and joining gene primer sets.



Method 1	Method 2	IGHV SHM Spearman Concordance Value
Sanger Sequencing	Oncomine BCR-IGH LR Assay (FR1-C)	0.849

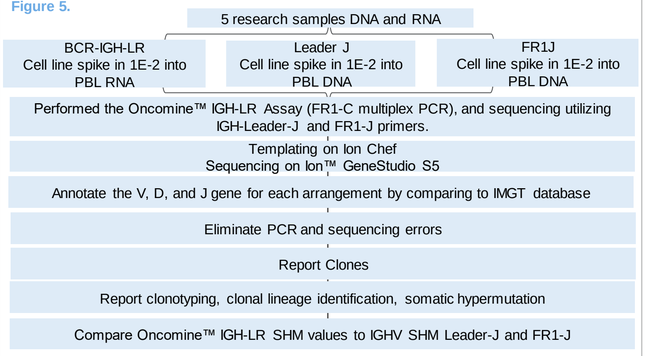
Figure 4. & Table 2. High concordance when comparing the IGHV SHM frequencies between BCR IGH-LR assay with sanger sequencing.

METHODS

Experimental Design and Informatics Workflow

Figure 5. Experimental Study. Libraries were prepared using the Oncomine BCR IGH-LR Assay from total RNA, the Oncomine™ IGHV SHM Leader J and FR1-J assay from genomic DNA extracted from peripheral blood spiked with lymphoma cell line genomic DNA or total RNA to a frequency of 10E-2 by mass ratio. Libraries were sequenced via the Ion GeneStudio™ S5 System followed by Ion Reporter analysis to identify clonotypes and evaluate B cell clone frequencies.

Figure 5.



RESULTS

Correlation between Ion Oncomine™ BCR IGH LR Assay and Oncomine Comprehensive™ IGHV SHM Assays Leader-J and FR1-J

Cell Line	SHM Frequency measured by IGH-LR (1)	SHM Frequency measured by IGH-LR (2)	SHM Frequency measured by Leader-J Assay (1)	SHM Frequency measured by Leader-J Assay (2)	SHM Frequency measured by FR1-J Assay (1)	SHM Frequency measured by FR1-J Assay (2)
MM.1R (CRL-2975)	---	---	1.7	1.7	2.2	2.2
JVM2	0.8	0.9	0.7	0.7	0.9	0.9
BDCM	5.7	5.7	5.1	5.4	5.7	5.7
Pfeiffer	2.2	2.2	1.7	1.7	2.2	2.2
GA-10	---	---	6.1	6.1	---	---
TMM	9.1	9.1	7.4	7.4	9.1	9.1

Table 3. Both RNA and DNA input assay workflows were able to correctly determine the SHM status of all rearrangements tested. IGHV SHM values were highly concordant between both RNA and DNA approaches. SHM values derived from FR1 targeting variable gene primers delivered concordant results compared to leader targeting variable gene primers when using DNA input across a wide range of SHM frequencies tested.

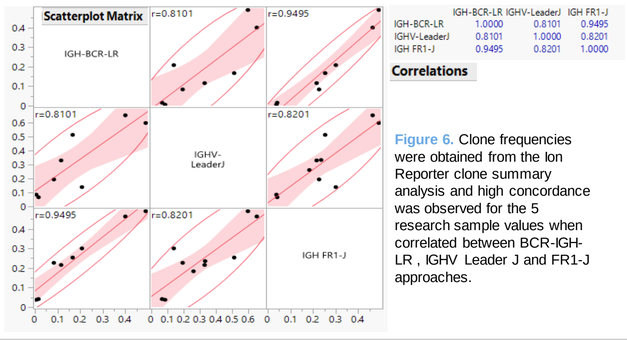


Figure 6. Clone frequencies were obtained from the Ion Reporter clone summary analysis and high concordance was observed for the 5 research sample values when correlated between BCR-IGH-LR , IGHV Leader J and FR1-J approaches.

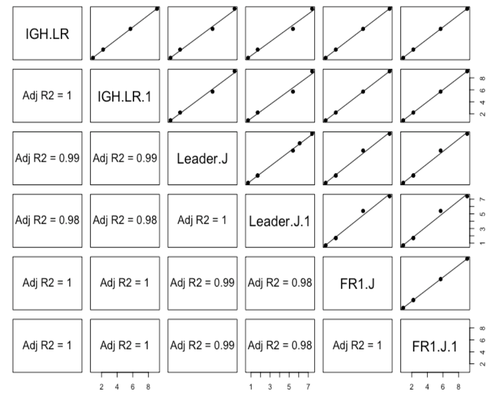


Figure 7. High concordance was observed when comparing SHM frequency values for 5 selected research cell lines that are correlated with an R^2 value of greater than 0.9 in comparison to the values derived from the IGH-LR assay.

CONCLUSIONS

These results support the ability of highly multiplexed long-read NGS assays to accurately quantify SHM in either DNA or RNA samples. Concordant results were shown between FR1 and Leader targeting primers using DNA input show the utility in both priming locations. RNA based NGS methods benefit from lower sample requirements as well as the addition of isotype (and subtype) identification, opening new research areas for study of the B cell immune repertoire.

REFERENCES

1. Huet, R., et al. *Leukemia* (**2020**) 34: 2257–2259; https://doi.org/10.1038/s41375-020-0716-1
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